**P548/M548 Final Miniproject**

**Due 4/29/15. Please also prepare a brief talk to present your results on 4/29/15**

**This week we introduced nutrient limitation of tumor growth in addition to our previous exploration of limitation of space availability.**

**I will use arbitrary names to denote the parameters (you can change them to whatever you find convenient).**

Some of the studies may require that you use a larger cell lattice than before. You can rough-out your model on smaller lattices and then run on larger lattices for “production runs.” You may find that lattice sizes of 128 x 128, 256 x 256, etc…. run faster. You may also want to use the multicore and GPU capabilities of CC3D to speed up your simulations (see CC3D manuals), but always run a simulation without this acceleration to make sure you have not introduced any artifacts.

Basic Tumor Evolution Model: A number of global parameters affect the availability of nutrients to the cells in the tumor: The rate of secretion (Glucose\_Secretion\_Rate) of the limiting nutrient (which we have called Glucose [Glu]) by the medium, the rate of diffusion of Glu (D\_Glucose), the background decay rate of Glu (Decay\_Glucose). The percentage of Glucose the cells take up (cell.Percentage\_Uptake) also determines the distance to which Glu can penetrate into a solid tumor.

In our simple model from this week, the response of the cells to the available Glu depends on three main factors, the basal metabolism of the cell (cell.basal\_Metabolism) we defined the available Glu (available\_Glu=Glu(cell.center\_of\_mass)\*cell.Percentage\_Uptake-cell.basal\_Metabolism). If available\_Glu < 0 we had the cell become necrotic and if available\_Glu > 0 we defined the rate at which the cell converts to cell mass using a Michaelis function with cell.target\_volume+=cell.conversion\_Efficiency\*available\_Glu(cell.glucose\_Saturation+available\_Glu)

1. First modify the code that you have been using so that you have periodic boundary conditions on the cell lattice and the Chemical Field lattice (look up how to do this in the manual). -CHECK
2. Now explore the effects of nutrient limitation by adjusting the parameter Glucose\_Secretion\_Rate. For each value run the simulation five or six times and see what happens. As you make Glucose\_Secretion\_Rate bigger you should see that the typical tumor mass grows. For many values, you may find that the tumor tends to die out when all the tumor’s stem cells die. For some values, the tumor may tend to split into multiple separate tumors.
3. Pick a couple of values of Glucose\_Secretion\_Rate which have different basic behaviors. Now vary D\_Glucose. Note that changing D\_Glucose does not change the availability of Glucose to the tumor, just its distributon. Does increasing D\_Glucose change the typical size of tumor clusters? Does it change the typical lifetime of the stem cells?
4. Now change cell.Percentage\_Uptake as in 2). What do you see? You may have to adjust the terms in your Michaelis function so that the typical growth rate of the cells stays roughly the same. What would you predict the relationship between these terms needs to be to keep the pattern the same?
5. Find sets of parameters for which you obtain a single long-lived tumor and others for which you obtain multiple separated tumors. Run 10 replicas of each and study the pattern of survival and tumor size.

In reality, cells store energy and don’t die immediately when they enter a nutrient deprived region. Define for each cell a cell.stored\_Glucose term and a cell.death\_Threshold term. You will have to play a bit to determine the appropriate initial values for cell.stored\_Glucose. Now change your growth function a little, so that at each MCS the cell.stored\_Glucose+=available\_Glu-cell.basal\_Metabolism. Add a new parameter cell.growth\_threshold > cell.death\_Threshold. If .stored\_Glucose> cell.growth\_threshold, then increase the cell’s target volume by an amount depending on cell.stored\_Glucose-cell.growth\_threshold. Think about what a reasonable form for this functional dependence might be. Remember to subtract any Glucose used for growth from the cell.stored\_Glucose.

1. Repeat steps 1-4 until you have a well behaved version of the tumor.

Tumor cells tend to alternate between proliferating and quiescent states. You can implement these states as cell types where the cells switch from proliferating🡪quiescent after a division if the cell.stored\_glucose<cell.quiescence\_threshold. Cells switch from quiescent🡪proliferating if cell.stored\_glucose>cell.proliferating\_threshold. The quiescent cells can store glucose, but never use the stored glucose to grow. You will have to have your cells carry multiple copies of their parameters (those for proliferating and quiescent stem cells and those for proliferating and quiescent somatic cells). You can make these values equal between types to being with, but may then want to define the default basal metabolism of quiescent cells to be lower than that of proliferating cells. You might also look in the literature to see if the values should differ between stem and somatic cells (ideally, you should give some biological reason for your choices).

1. Adjust your parameters so that your tumor(s) consist of a core of necrotic cells surrounded by a layer of quiescent cells with a thin coating of proliferating cells on the surface of the tumor. What parameters control the thicknesses of the various layers? You may find it helpful to define a necrotic cell type which does not consume Glucose, but which has a target volume that decreases slowly to zero (instead of having the volume go abruptly to zero as we defined it before).

Now allow your growth parameters to evolve.

1. First allow the thresholds for cell switching between proliferation and quiescence to evolve. What do you find? How does the rate of evolution depend on the availability of Glucose and the Glucose diffusion rate?
2. Now allow the cells to adjust their cell.perecentage\_Uptake and cell.growth\_Threshold to evolve. Make sure you don’t cheat by allowing the cells to convert Glucose to target volume more efficiently! There is no free lunch.

**Adhesion Evolution:** We originally defined the adhesivities between our cell types on the basis of cell type. Switch the contact energy of the cells to AdhesionFlex in your CC3DML code. This change will allow each cell to have a unique adhesivity with its neighbors. Define a cadherin adhesion molecule that causes tumor cells to stick to each other and an integrin adhesion molecule that causes cells to adhere to the medium (stroma). Cells with high integrin and low cadherin should disperse into the medium (stroma), cells with low integrin and high cadherin should stay attached to the tumor. Cells with intermediate levels of both integrin and cadherin should stay attached to the tumor but remain on its surface. Check that these expectations are borne out in your simulations and note the typical values of integrin and cadherin for which they occur.

1. Adjust the base adhesivities so that the tumor remains compact and does not differ substantially from the tumors you generated in 6).

Now you are free to begin exploring somatic evolution more seriously. You should verify that cells which move to the center of a tumor have a high probability of dying. Thus cells will do better when they stay on the surface of the tumor.

1. Allow your cell’s integrin and cadherin levels to evolve. You will have to define reasonable evolution rules for these values. Run a number of replicas. What happens to the mean integrin and cadherin levels of the cells? Are there qualitative changes in tumor morphology (e.g. death, splitting, sudden growth,….) that correlate with specific patterns of adhesion molecule change? Hint: You should find that integrin levels grow and cadherin levels decrease, but the pattern will depend on the pressures you apply.
2. Estimate the typical gradient of Glucose in your tumor and measure the typical rate of evolution of the adhesion parameters. Now change the gradient without changing anything else in your model (the easiest way is by adjusting D\_Glucose). Plot the rate of evolution of the adhesion parameters vs the gradient magnitude. You should find that the evolution is slow for very steep and very shallow gradients and maximal for some intermediate value. Why? How does this gradient compare to the length scale of the cells? Do other parameters have a maximal rate of evolution for the same gradient? Why?

Try to summarize what you have learned so far. What factors cause the tumor to evolve most rapidly? What factors cause it to grow the largest? What factors cause it to tend to stay compact? To split into multiple small clusters? To disperse into single cells or few cell clusters? When do tumor clusters die spontaneously? Why?

Repeat our simulations where you periodically remove a substantial fraction of the tumor by “surgery” “raditation” or “chemotherapy”. Make sure you time the intervals between treatments to be a realistic multiple of the typical cell cycle times in your tumor (you could assume that the fastest cell cycling you see corresponds to about 48 h). Do these treatments enhance or impede the evolution of the tumor? What aspects of the tumor change the most due to treatment? Could you change the intensity/duration/interval of the treatment to increase the probability of success?

**Extensions:** If you want to go further, you could explore a number of alternative mechanisms of tumor progression:

**The Warburg Effect**: Cells normally change their balance between aerobic and anaerobic metabolism depending on local O2 levels. Many cancer cells have a higher rate of anaerobic metabolism for the same O2 level than normal cells.

1. To study this effect, add a second chemical field O2 produced by the medium (it should diffuse faster than the Glucose field). As for Glu, each cell needs to take up a percentage of the O2 available to it. Give each cell a cell.Warburg parameter that defines the threshold between aerobic and anaerobic metabolism in terms of the O2 level. Define a Hill functions increasing as a function of O2, with an inflection point at cell.Warburg. For O2 << cell.Warburg the function should be a cell.base\_aerobic and for O2>>cell.Warburg the function should be 1-cell.base\_aerobic. This function defines the fraction of aerobic and anaerobic metabolism for each O2 level. Modify your growth functions as follows: The efficiency of conversion of Glu🡪Target volume for anaerobic metabolism is roughly 50% of that for aerobic metabolism. Add a death term that kills cells with an O2 level below a threshold. The larger cell. Warburg the greater the threshold (Warburg cells are more resistant to O2 deprivation). Allow cell.Warburg to evolve. What happens? Do slower growing (high Warburg) cells win out? Why?

**Explicit Stromal Cells:** We have been modeling the surrounding tissue as effectively unoccupied space through which cells are free to move. In reality, this space is full of cells and ECM which can promote or inhibit cancer cell movement.

1. Replacing medium with a **stromal** cell type which represents normal cells. Except for having a fixed target volume, these cells are otherwise identical to medium in their properties. Include a pressure limitation on tumor cell growth as we did early on. Now, the tumor is space limited as well as glucose limited.
2. When cells undergo anaerobic metabolism, they secrete lactic acid, which is toxic to cells. Add a lacticAcid field, which is secreted by cells undergoing anaerobic metabolism and taken up at a low rate by all cells. Each cell has a threshold such that it dies if the level of lactic acid exceeds that threshold. Initially all calls have the same threshold (both stromal, stem and somatic cells). Allow the threshold to evolve in the stem and somatic cells (you should include a trade-off function where a higher threshold increases the cells basal metabolism). What happens? How does this evolution change for steeper tradeoff functions?
3. Some tumor cells also produce Matrix-Metalloproteases which degrade the surrounding ECM and can kill stromal cells. You can model MMPs by including a small probability of their production and a variable rate of production when produced. As always, producing MMPs increases the basal metabolism of the cell, reducing its maximum growth rate. MMPs can function in two ways, by direct contact and secretion. In the former case, contact between an MMP producing cell and stromal cells reduces the target volume of the contacted stromal cells. In the latter case, the secreted MMPs are absorbed by the stromal cells, again reducing their volumes). Assume that only somatic cells can produce MMPs and compare the effects of contact and secreted MMPs.
4. What happens if the MMPS can kill tumor cells as well as stromal cells? You can assume that an MMP producing cell can kill only non-MMP-producing cells, or you can have an evolvable MMP resistance (where greater resistance increases basal metabolism).

**Growth Factors and Common Goods Games**: Many tumor cells initially require one or more growth factors secreted by the stromal tissue to grow. As before, add a new field, growth factor, produced by the stromal tissue (Ideally you should include a small number of randomly dispersed stromal cells which secrete the growth factor rather than having all of the medium secrete it—that way, if the tumor kills these cells off it can’t recover the growth factor just by dying back). The tumor cells take up this growth factor just as they do glucose or O2. However, the cells cannot enter a proliferating state unless they have a sufficient level of growth factor. What happens? If a cells could evolve so it didn’t need the growth factor, it would clearly have an advantage. However, while this does happen, cancer cells often solve this limitation in a different way. Suppose that the cancer cells have a small probability of evolving the ability to produce and secrete this growth factor and the level of production is an evolvable quantity. This production makes the tumor less dependent on the availability of the factor from the stromal tissue. Allow the cells’ production of growth factor to evolve. Make sure that the production of growth factor has a significant cost (i.e. it increases the basal metabolism of the producing cell). Also—make sure that ONLY somatic cells can produce growth factor. This sets up a common-goods conflict. Cells that don’t produce the growth factor can “cheat” by using factor produced by other cells. In addition, stem cells are now dependent on their somatic offspring. Explore these conflicts. If you periodically supply a “drug” that diffuses from the stromal tissue and interacts with the growth factor to inactivate it, how does it affect the evolution of the tumor? Remember that the inactivation is never perfect and that it uses up the drug, so regions with high levels of growth factors should have some left, even at high drug doses. You might want to add a cell variety which doesn’t need the growth factor, but has a much lower rate of growth than those that do.

**Immune System Killing and Resistance**: Stromal tissue contains immune cells which kill the vast majority of premalignant cells before they cause problems. We won’t try to model the complexity of the real immune system, but we can explore some basic effects of it on evolution. A simple model of the immune system says that the probability of a tumor cell being killed is proportional to its contact area with stromal cells/medium (look up how to turn on this measurement and add it to your killing stoppable). If the immune system’s killing effect is strong, it favors compact round tumors, if weak, tumors can disperse more easily.

1. See how the effect of the immune system modulates changes in cell adhesivity (as investigated before).
2. Lactic acid is an immune-system inhibitor. Include a modulation term in your immune killing function so that the probability of cell death depends on both contact with medium/stroma and lactic acid levels. What happens?
3. Some tumor cells also evolve the ability to produce immunosuppressive cytokines. Model their production just as you modeled growth factor production in the section above. Cells have a small probability of secreting a diffusible cytokine, which decreases the probability of immune killing. As before, only somatic cells can secrete cytokines and their production increases basal metabolism. What happens and how does it depend on the diffusion constant and efficacy of the cytokine?

**Time Variation of Nutrients and Motility:** In a vascular tumor, the availability of nutrients changes in time due to the formation of new blood vessels and the collapse of existing blood vessels. Define a number of source “vascular” cells scattered throughout the cell lattice which will act as sources for Glucose, O2, growth factors,….. You will have to adjust their density and secretion rates to match the basic patterns of your previous simulations. Check that the basic simulation works as before. You may have to move to a larger simulation (e.g. 512 x 512) to do this project.

1. Now assume that there is a probability per unit time that a source turns off or turns on (typical real time scales are on the order of weeks). How does this affect the evolution rates of your parameters (especially the cell motility, which you can measure by tracking the displacement of cells in time)? How does the rate of evolution of motility depend on the spacing between the source sites (measured in units of the diffusion length) and the time that the source remains on/off?
2. In reality, the probability of turning off depends on the local hydrostatic pressure. Add a function which modulates the probability based on the local hydrostatic pressure. What happens?
3. Cells can move actively as well as passively. You can increase intrinsic cell motility in a number of ways, but the easiest one is through explicit forces (look up how to implement these in the CC3D manuals). As usual, a tumor cell can evolve an active motility, which is represented by applying an explicit force to the cell in a random direction for a fixed amount of time. The greater the active motility, the higher the basal metabolism of the cell. You should compare the cases where only somatic cells are actively motile and when both stem and somatic cells are actively motile. For a given range of motilities, what is the rate of evolution of the motility as a function of the length and time scale of the regions lacking nutrient sources. You should find that the evolution is slow for very small and very large regions and for very slow and very fast switching. What is the regime that has the fastest switching and why? Simulate the action of an antiangiogenic, which increases the rate at which sources disappear and decreases the rate at which they disappear. How does this therapy affect the rate of evolution of invasive behavior? How could you use this observation to develop alternative treatments for cancers?